# HYDROGEN PEROXIDE-MEDIATED MODULATION OF GERMINATION RATE AND SEEDLING VIGOR IN *Psophocarpus tetragonolobus* (L.) DC.

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Winged bean (*Psophocarpus tetragonolobus* (L.) DC.), a tropical legume rich in protein, micronutrients, and antioxidants, holds significant potential for food security and sustainable agriculture due to its nutritional value and nitrogen-fixing ability. However, its cultivation is limited by low germination rates primarily caused by hard seed coats and dormancy. This study investigated the effect of Hydrogen peroxide ($H_2O_2$) as a pretreatment agent for modulating germination rate and enhancing seedling vigour in *P. tetragonolobus* (variety – SLS 44). Seeds were subjected to six concentrations of $H_2O_2$ (0.5%, 1%, 2%, 3%, 5%, and 7%), using a completely randomized design with a negative control treatment. Parameters assessed included mean germination percentage, mean lateral root count, mean root, and shoot lengths, measured at 5- and 10-days post-treatment, along with the cumulative growth rate. The results revealed that the 3% $H_2O_2$ treatment was the most effective, yielding the best outcomes for both seed germination and seedling development. After 10 days, seeds treated with 3% $H_2O_2$ exhibited the highest germination percentage (71.11%) and significantly enhanced root and shoot lengths (39.66±0.92 mm and 28.49±1.37 mm, respectively). Mean lateral root count (5 roots) and cumulative growth rate (7.13 mm/day) were also highest at this concentration. Statistical analysis via ANOVA confirmed significant effects of treatment day on all variables ($p < 0.05$), and of $H_2O_2$ concentration on mean germination percentage and mean root length. These findings suggest that hydrogen peroxide acts not only as a dormancy-breaking agent but also as a metabolic enhancer, likely modulating hormonal balances by increasing gibberellin and reducing abscisic acid levels. In conclusion, Hydrogen peroxide ($H_2O_2$) pretreatment at 3% concentration is a cost-effective way to break seed dormancy, boost germination, and enhance early growth in winged bean. Further studies are needed to confirm its reliability across various commercial $H_2O_2$ formulations, experimental designs, and varying agro-climatic conditions.

*Keywords*: cumulative growth rate; dormancy; germination rates; hard seed coat; Hydrogen peroxide; *Psophocarpus tetragonolobus* (L.) DC.

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## INTRODUCTION

Winged bean (*Psophocarpus tetragonolobus* (L.) DC.), a tropical legume from the Fabaceae family, is a highly nutritious and versatile crop native to Southeast Asia [1]. It is valued for its edible pods, seeds, and leaves which are rich in protein (up to 35%), fats, antioxidants, and micronutrients [2]. The plant thrives in warm climates (25–35°C) with adequate moisture and well-drained soils, and it plays a significant role in sustainable agriculture due to its nitrogen-fixing ability [3]. Despite its potential, winged bean cultivation faces challenges, particularly in seed germination, which is often hindered by hard seed coats and dormancy [4].

Hydrogen peroxide ($H_2O_2$), a reactive oxygen species (ROS), is a pale blue liquid with strong oxidizing properties [5] [6]. It decomposes into water and oxygen, making it useful in various applications, including seed scarification. In seed germination, $H_2O_2$ acts as a signaling molecule, breaking dormancy by weakening the seed coat, promoting the oxidation of inhibitory compounds, and activating metabolic pathways. It enhances gibberellin biosynthesis while reducing abscisic acid (ABA) levels, thereby facilitating the transition from dormancy to germination. Additionally, $H_2O_2$ pretreatment improves antioxidant defense mechanisms and mobilizes nutrient reserves, leading to higher germination rates and vigorous seedling growth [7].

This study explores the use of hydrogen peroxide as a scarification agent to overcome the germination barriers in winged bean seeds. By examining the physio-chemical effects of $H_2O_2$ on seed coat permeability and metabolic activation, the research highlights its potential to enhance germination efficiency and early seedling development. Since Hydrogen peroxide has the potential to significantly affect the early seedlings developmental process, for both farmers and researchers, the findings of this research could provide valuable insights into improving the seed germination of *P. tetragonolobus* and its cultivation.

## METHODOLOGY

### *Research design*

Seeds of *P. tetragonolobus* (variety – SLS 44) were sourced from the Department

of Agriculture, Peradeniya, Sri Lanka, and selected based on uniformity in size, shape, and colour. A viability test was performed to ensure consistency, retaining only seeds that sank in water. Seedbeds were prepared in sterilized Petri dishes, each lined with cotton wool and filter paper, and sterilized at 180°C for 2 hours to prevent microbial contamination. Hydrogen peroxide treatments included six concentrations: C1 – 0.5%, C2 – 1%, C3 – 2%, C4 – 3%, C5 – 5%, C6 – 7%. These were prepared by diluting analytical-grade $H_2O_2$ (50% v/v, Merck brand) with distilled water and stored in dark bottles to prevent light-induced decomposition. Seeds were grouped accordingly (45 seeds per treatment), surface-sterilized with 1% sodium hypochlorite for 1 minute [8], rinsed 2-3 times with sterile distilled water, then soaked in their respective $H_2O_2$ solutions for 6 hours [9]. After soaking, seeds were rinsed with sterile distilled water to remove residual peroxide. The experiment followed a completely randomized design (CRD) with the six treatments (C1–C6) and a negative control (NC – sterile distilled water), each replicated three times with 15 seeds per replicate. Seeds were placed in Petri dishes with 2 ml of distilled water and maintained in dark, at room temperature ($25^0$C). Daily monitoring ensured consistent moisture and minimal exposure to temperature changes and light.

***Evaluation of the effect of Hydrogen peroxide***

Collection of data and measurements were conducted at two distinct time points: after 5 days and after 10 days of hydrogen peroxide treatment. At each interval, the following parameters were assessed: mean germination percentage, mean lateral root count, mean root length, and mean shoot length. Cumulative growth rate was calculated based on observations over 5-days period (from $5^{th}$ day to $10^{th}$ day) using the following equation. This comprehensive approach provided insights into the effects of hydrogen peroxide on the experimental units.

Cumulative growth rate; $G_C$

$G_C$ = [(Mean final length of shoot at $10^{th}$ day - Mean initial length of shoot at $5^{th}$ day) + (Mean final length of root at $10^{th}$ day - Mean initial length of root at $5^{th}$ day)] / 5 days

Analysis of variance (ANOVA) was conducted using Minitab version 22.3.0. (Minitab LLC., 2021) to assess the effects of $H_2O_2$ concentrations and time (day) on mean germination percentage, mean root length, and mean shoot length. A p-value of less than 0.05 ($p<0.05$) was considered statistically significant.

## RESULTS AND DISCUSSION

This study investigated how hydrogen peroxide pretreatment significantly affected the germination and root growth of *P. tetragonolobus* seeds. Germination quality was assessed based on mean lateral root count, mean root length, mean shoot length, mean germination percentage, and cumulative growth rate (Table 01, Figure 01).

*Table 01: Treatments and observations in seed germination, root and shoot growth and lateral root count of developing seedlings, $G_C$; cumulative growth rate*

| Treatment | [$H_2O_2$ ] % v/v | Mean germination percentage % | | Mean root length (Length ±Standard error; SE) mm | | Mean shoot length (Length ±SE) mm | | Mean lateral root count | $G_C$ (mm/day) |
|---|---|---|---|---|---|---|---|---|---|
| | | 5th day | 10th day | 5th day | 10th day | 5th day | 10th day | 10th day | |
| NC | 0 | 8.89 | 37.78 | 13.89±1.45 | 17.11±2.11 | 2.67±1.20 | 13.00±5.90 | 2 | 2.71 |
| C1 | 0.5 | 33.33 | 53.33 | 15.68±1.01 | 22.77±2.40 | 5.94±2.14 | 13.53±1.87 | 4 | 2.93 |
| C2 | 1 | 62.22 | 66.67 | 11.93±2.75 | 28.24±0.64 | 6.56±1.82 | 12.08±2.06 | 3 | 4.37 |
| C3 | 2 | 57.78 | 64.44 | 19.86±1.44 | 36.01±2.27 | 6.73±1.51 | 24.77±3.91 | 4 | 6.84 |
| C4 | 3 | 66.67 | 71.11 | 23.67±0.68 | 39.66±0.92 | 8.85±1.61 | 28.49±1.37 | 5 | 7.13 |
| C5 | 5 | 42.22 | 53.33 | 21.43±0.78 | 37.29±2.69 | 8.62±1.57 | 23.57±5.34 | 4 | 6.16 |
| C6 | 7 | 48.89 | 64.44 | 18.71±0.88 | 24.62±3.46 | 8.44±0.55 | 23.68±3.05 | 4 | 4.23 |

## *Statistical Analysis*

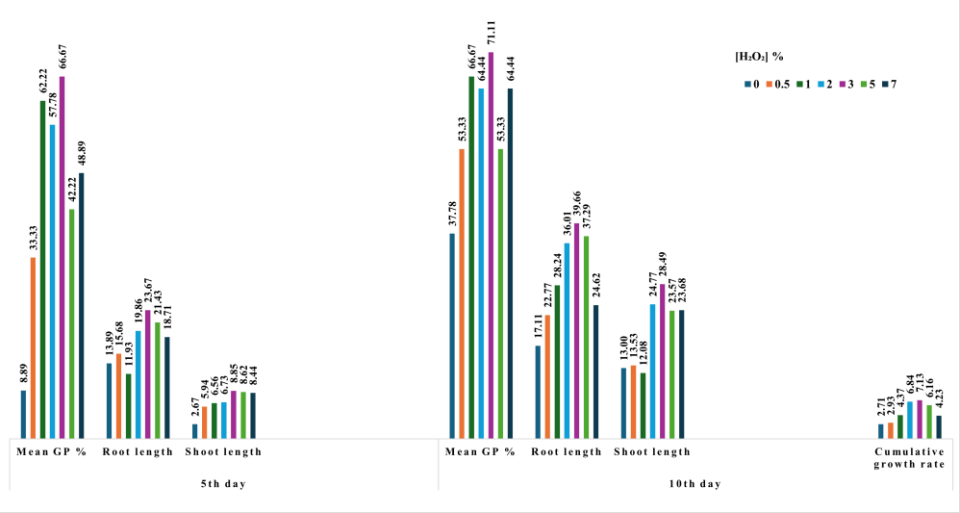
*Table 02 – p-value of ANOVA results*

| | Mean germination percentage | | Mean root length | | Mean shoot length | |
|---|---|---|---|---|---|---|
| | Day | Concentration | Day | Concentration | Day | Concentration |
| p | 0.009 | 0.004 | 0.002 | 0.050 | 0.001 | 0.140 |

### *Optimal effect of the moderate $H_2O_2$ concentration*

The results clearly showed that 3% $H_2O_2$ concentration was optimal for promoting germination and seedling vigour. After ten days, seeds exposed to 3%

hydrogen peroxide ($H_2O_2$) developed an average of five lateral roots, which was the highest count observed. This marked an increase compared to the control group, which produced only two roots without $H_2O_2$ treatment. In addition, the highest mean root length (23.67±0.68 mm in 5$^{th}$ day and 39.66±0.92 mm in 10$^{th}$ day) and mean shoot length (8.85±1.61 mm in 5$^{th}$ day and 28.49±1.37 mm in 10$^{th}$ day) were observed in the 3% $H_2O_2$ treatment. These results suggest that $H_2O_2$ breaks seed dormancy, enhances water uptake, and activates metabolic activities necessary for germination and root growth. In particular, there is evidence that $H_2O_2$ acts as a downstream signalling molecule in the auxin pathway, altering hormonal balance by increasing gibberellin (GA) levels and decreasing abscisic




acid (ABA) levels [10].

*Figure 01: Mean germination percentages, mean root and shoot lengths in 5$^{th}$ day and 10$^{th}$ day and cumulative growth rate*

***Concentration-dependent effect and the "oxidative window" hypothesis***

Study results revealed that the influence of hydrogen peroxide on development of seedlings is strongly concentration-dependent. Although a 2% $H_2O_2$ treatment produced notable improvements, the 3% concentration emerged as the most effective in enhancing overall germination and seedling growth. Lower concentrations, such as 1%, stimulated growth relative to the control but failed to generate the oxidative stimulus required for optimal results. In contrast, higher concentrations, particularly 5% and 7%, resulted in reduced germination, inhibited root elongation, and a general decline in seedling vigour, likely due to cellular or seed damage [11]. These results are consistent with the "oxidative window" hypothesis proposed by Bailly *et al*. [12], which suggests that moderate levels of reactive oxygen species (ROS) play a promotive role in germination and early growth, while high levels can damage cellular components, reduce seed viability, and inhibit of growth.

***Germination percentage and cumulative growth rate***

Hydrogen peroxide pretreatment significantly enhanced the mean germination percentage of *P. tetragonolobus* seeds. After five days, seeds treated with 3% $H_2O_2$ exhibited the highest germination rate at 66.67%, a substantial improvement over the 8.89% observed in the control treatment. By the tenth day, this increased further to 71.11%. Additionally, the 3% concentration achieved the highest cumulative growth rate of 7.13 mm/day, underscoring its effectiveness in promoting overall seedling growth and vigour. These findings align with previous research highlighting $H_2O_2$ as a potent germination stimulant in various plant species [13], although optimal concentrations may differ depending on the species.

## CONCLUSIONS/RECOMMENDATIONS

Overall, this study concludes that $H_2O_2$ pretreatment is a highly effective approach for improving seed germination and root development in *P. tetragonolobus*. Hydrogen peroxide at a 3% concentration consistently produces the most favourable outcomes, significantly enhancing germination quality and seedling vigour. These findings highlight the potential of $H_2O_2$ application as a practical and low-cost method to optimize early-stage growth in winged bean cultivation, offering promising implications for improved crop establishment and agricultural productivity. Furthermore, the investigators recommend repeating the study using various commercial $H_2O_2$ brands, experimental setups, and different environmental conditions to comprehensively assess the consistency and broader applicability of the observed effects.

## ACKNOWLEDGEMENTS

The investigators/researchers sincerely extend their gratitude to the laboratory staff of the Department of Botany at the Open University of Sri Lanka, Kandy Regional Centre, as well as to the colleagues whose contributions and assistance were invaluable in many ways.

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